

# Utilization of spent brewer's yeast for supplementation of distillery corn mashes

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The aim of the study was to use spent brewer's yeast biomass (SBY) as a nutrient adjunct of distillery type corn mashes to improve the process of ethanol fermentation by yeast *Saccharomyces cerevisiae*. There were prepared corn mashes with raw material loading at 20% ww. with SBY addition at solids loadings of 0 (control); 0.1; 0.5; 0.7; 1.0; 3.0 and 5.0% ww. The obtained mashes were inoculated with yeast and subjected to batch fermentation for 72 h. It was observed that supplementation of corn mashes with SBY improved the process of fermentation. The consumption of sugars and production of ethanol by yeast in supplemented mashes was accelerated and the overall ethanol yield was improved by 6.5 to 11% depending on the amount of added SBY. It was also observed that the fermentation could be shortened by 24 h in mashes enriched with SBY.

**Keywords:** ethanol fermentation, corn mash, spent brewer's yeast, *Saccharomyces cerevisiae*.

## INTRODUCTION

Intensification of fuel ethanol production is currently of high importance due to depletion of fossil fuels. To improve the competitiveness of fuel ethanol in relation to fossil fuels it is necessary to obtain the low cost of the final product. The reduction in the price of fuel ethanol can be achieved by the improvement of productivity without major modification of the production process what currently is the field of study by many researchers and manufacturers. The best way to improve the process of ethanol fermentation catalyzed by yeast (*Saccharomyces cerevisiae*) is the production media supplementation with proper nutrients. The most important nutrient is assimilable nitrogen which is crucial for cell multiplication and enzyme synthesis<sup>1</sup>. Other nutrients essential for fast yeast metabolism are vitamins (biotin, thiamine, pantoic acid)<sup>2</sup>, fatty acids<sup>3</sup> and minerals<sup>4</sup>. In industrial environment inorganic salts (i.e. ammonium sulphate) or urea are commonly used for media enrichment with assimilable nitrogen, however, as discovered by Junior and others<sup>5</sup>, mineral salts and urea are not sufficient for achieving high ethanol yield. To achieve the improved fermentation complex nutrient source containing, besides assimilable nitrogen, vitamins, fatty acids and trace minerals has to be used. It is also important that media supplementation source was inexpensive and highly available. Earlier studies have shown that the use of food industry wastes like soy skim milk<sup>6</sup> or dried distiller's grains with solubles<sup>7</sup> had improved ethanol fermentation yield and rate.

One of the promising media supplements could be spent brewer's yeast resulting from the production of beer. In the course of beer production yeast is used several times for fermentation but when cell viability is decreasing it must be replaced with fresh culture. The amount of spent yeast produced by the brewery industry can reach even 2.5% of beer produced<sup>8</sup>. Most commonly spent yeast is used as protein-rich animal feed, although its share in the feed is limited due to its bitter taste. Other potential applications for spent brewer's yeast utilization are: pharmaceuticals production<sup>9</sup>, extracts production<sup>10</sup> and  $\beta$ -glucan extraction<sup>11</sup>. Besides the production of

industrial products, spent brewer's yeast has a great potential for improving biotechnological processes. Spent brewer's yeast biomass is characterized by rich chemical composition in which protein, vitamins (mainly niacin and thiamine) and minerals (potassium and calcium)<sup>12</sup> are abundant. Yeast extracts are widely used in a laboratory routine for the preparation of growth and fermentation media for various microorganisms, but their industrial use is limited due to high prices. For industrial purposes spent yeast should be inactivated at a little cost. For the purpose of fuel ethanol production, inactivation of spent yeast biomass as a separate process is not economically appropriate. During the production of ethanol, inactivation of spent brewer's yeast can be achieved in the course of the mashing process where high temperature and action of hydrolytic enzymes occur simultaneously.

The purpose of this study was to use spent brewer's yeast as a nutrient supplement for distillery type corn mashes and to assess the course and yield of ethanol fermentation of corn mashes supplemented with spent brewer's yeast at varied doses.

## EXPERIMENTAL

### Raw materials

The corn grain of Susann cultivar harvested during the 2011 season was kindly provided by Saaten-Union. The grain was ground using Brabender Rotary Mill (Brabender, Germany) with 1.0 mm sieve inserted. The obtained meal was sieved through 630  $\mu$ m screen and larger particles were ground again using WZ-1 (ZBPP, Poland) knife mill and sieved through the same screen. The obtained corn meal was stored in airtight jars at room temperature until used. Starch content in corn meal was measured using Evers polarimetric method<sup>13</sup> and moisture content was measured using WPS-50 weighing-dryer (Radwag, Poland). The content of starch in material was 75.79% ww. and moisture content was 8.91% ww. Spent brewer's yeast biomass (SBY) was obtained from a local brewery. The yeast were transported in cooling conditions and separated from residual beer by centrifugation using MPW-351R centrifuge (MPW

Med. Instruments, Poland) at 4500 rpm at 4°C for 15 min. The supernatant was rejected and yeast precipitant was washed with sterile distilled water to remove the residual dissolved solids and separated again. After that yeast biomass was stored at -20°C until used. Dry matter content in SBY was measured as mentioned above and it ranged 33.18% ww.

### Biological materials

Active dry distillery yeast strain *Saccharomyces cerevisiae* Ethanol Red was obtained from Fermentis, France. Prior to inoculation the yeast was rehydrated in sterile distilled water as mentioned earlier<sup>14</sup>. Commercial enzyme preparations: Termamyl SC DS (thermostable  $\alpha$ -amylase), SAN Extra L (glucoamylase), Neutrase 0.8 L (protease) and Viscoferm (blend of cellulase and  $\beta$ -glucanase) were obtained from Novozymes, Denmark.

**The mashing process** The mashing of raw materials was performed using LB-12 mashing apparatus (Lochner Labor+Technik GmbH, Germany). The corn meal loading was 20% ww. and SBY was 0 (control sample), 0.1; 0.5; 0.7; 1.0; 3.0 and 5.0% ww. Detailed diagram of mashing process is shown in Figure 1.

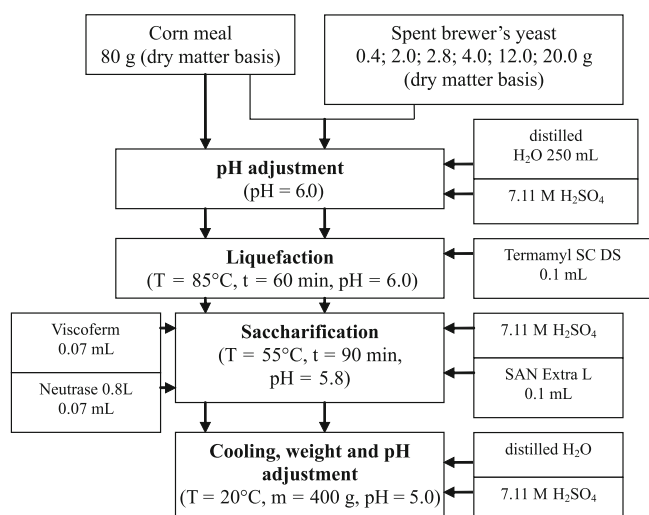


Figure 1. Detailed diagram of mash preparation

### Inoculation and fermentation

The obtained mashes were inoculated with 1 g kg<sup>-1</sup> of rehydrated *S. cerevisiae* Ethanol Red yeast. Then 200 g of mashes were transferred to 300 mL conical flasks and sealed with silicone stoppers with attached fermentation tube and sampling port as described previously<sup>4</sup>. The flasks were placed in a Type 357 water bath shaker (Elpin+, Poland) at 30°C and 50 rpm agitation speed for 72 h. Both mashing and fermentation experiments were performed in duplicate.

### Analytical methods

The fermentation process dynamics was assessed gravimetrically as the weight loss of the fermentation media at adequate time intervals<sup>14</sup>. Carbohydrate profiles (dextrins (DP 4+), maltotriose, maltose and glucose) of mashes and fermentation liquids, ethanol and glycerol were measured using high performance liquid chromatography (HPLC). The samples for chromatographic analyses were taken after the mashing process and every 24 h of fermentation. The samples were centrifuged (7000 rpm,

4°C, 10 min), clear supernatants were 5-fold diluted and filtered through 0.2  $\mu$ m nylon syringe filter and analyzed. The chromatographic analysis were performed using Prominence type chromatograph (Shimadzu, Japan) with Rezex ROA-Organic Acid H+ column (Phenomenex, USA) at following parameters: eluent – 0.005 M H<sub>2</sub>SO<sub>4</sub>, flow rate – 0.6 ml min<sup>-1</sup>, elution temperature – 60°C. The compounds were detected using RID-10 refractive index detector (Shimadzu, Japan) at 50°C. Integration of chromatograms was performed using Chromax 10 software (Pol-Lab, Poland).

On the basis of the obtained results kinetic parameters of fermentation were calculated (at 24 h time intervals): rate of sugars consumption (counted over glucose),  $R_s$  (1):

$$R_s = \frac{\Delta C_s}{\Delta t} \quad (1)$$

where:  $R_s$  – rate of sugars consumption [g L<sup>-1</sup> h<sup>-1</sup>],  $\Delta C_s$  – change in the concentration of sugars [g L<sup>-1</sup>],  $\Delta t$  – time interval [h]; degree of attenuation (counted over glucose),  $\alpha$  (2):

$$\alpha = \frac{C_{s_0} - C_{s_1}}{C_{s_0}} \times 100\% \quad (2)$$

where:  $\alpha$  – degree of attenuation [%],  $C_{s_0}$  – initial concentration of sugars [g L<sup>-1</sup>],  $C_{s_1}$  – concentration of sugars at adequate time interval [g L<sup>-1</sup>]; rate of ethanol production,  $R_p$  (3):

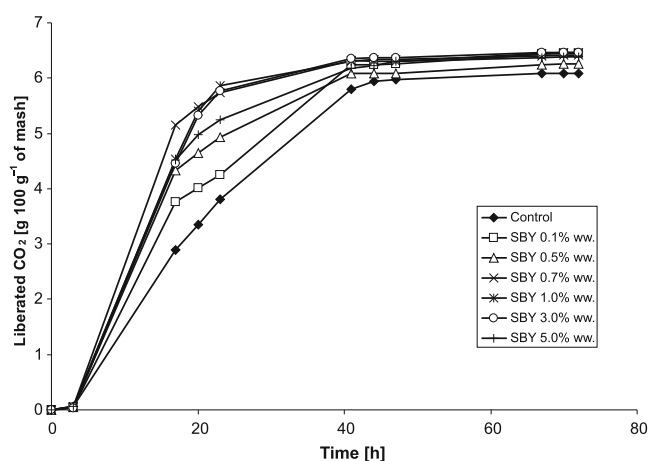
$$R_p = \frac{\Delta C_e}{\Delta t} \quad (3)$$

where:  $R_p$  – rate of ethanol production [g L<sup>-1</sup> h<sup>-1</sup>],  $\Delta C_e$  – change in the concentration of ethanol [g L<sup>-1</sup>],  $\Delta t$  – time interval [h]; practical ethanol yield,  $Y_p$  (%) on the basis of stoichiometric reaction where 100 g of starch is converted to 56.8 g of ethanol<sup>15</sup>.

The presented results are mean values obtained from two independent replications.

## RESULTS AND DISCUSSION

The rate of carbon dioxide liberation during ethanol fermentation is the key parameter for the estimation of the process course. During the fermentation of corn mashes supplemented with different doses of SBY a significant acceleration of fermentation was observed within the first day of the process (Fig. 2). It was observed that during the first 24 h of fermentation ca. 82–91% of total CO<sub>2</sub> was liberated in samples supplemented with 0.7% ww. and more of SBY, after the same time in the control sample only ca. 62% of gas was liberated. After 48 h of fermentation the amount of released carbon dioxide was similar in all studied samples. The amount of CO<sub>2</sub> liberated from fermenting mashes after the completion of fermentation was higher in supplemented samples (ca. 6.4 g 100 g<sup>-1</sup> of mash) in comparison to control (6.09 g 100 g<sup>-1</sup>). It was previously discovered that addition of assimilable nitrogen sources improved fermentation rate<sup>16</sup> and led to higher overall ethanol yield<sup>4</sup>. The higher rate of CO<sub>2</sub> liberation was the effect of faster adaptation of yeast cells to the fermentation environment in comparison to control sample, this fact is crucial for bacterial contamination prevention<sup>17</sup>.

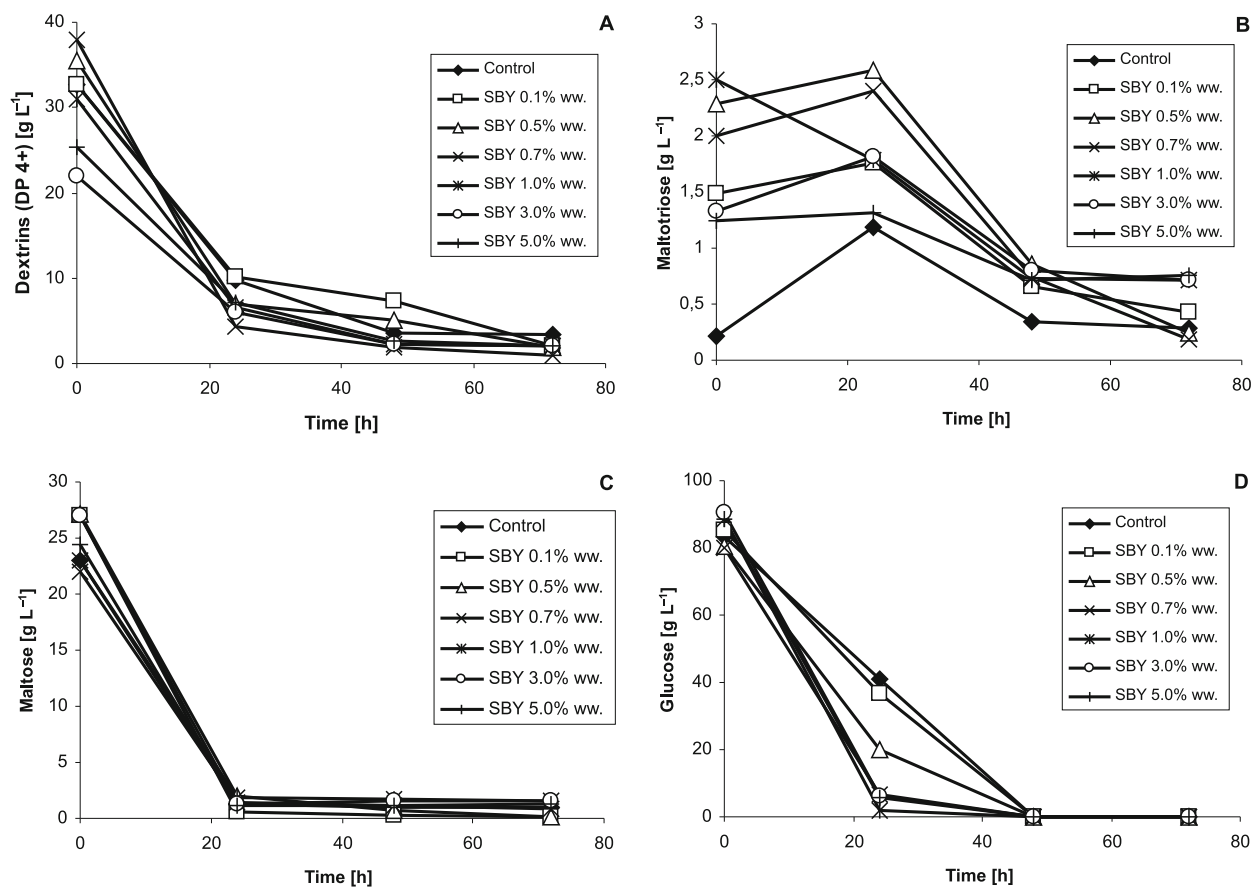


**Figure 2.** Dynamics of CO<sub>2</sub> liberation during fermentation of corn mash supplemented with spent brewer's yeast (SBY).

The initial concentration of oligomeric sugars (dextrins, maltotriose) in obtained mash was higher in samples supplemented with SBY (Fig. 3A and 3B). Most of the dextrins were hydrolyzed within the first two days of fermentation, and its final concentration ranged ca. 1–3 g L<sup>-1</sup>. In supplemented mash the initial concentration of maltotriose was much higher (1–2 g L<sup>-1</sup>) in comparison to control (0.2 g L<sup>-1</sup>). During the fermentation process its content in the fermentation media increased in every variant of the experiment within the first 24 h. In the later stages of fermentation, the concentration of maltotriose did not significantly change. At the beginning of fermentation the concentration of maltose ranged ca. 21–27 g L<sup>-1</sup> (Fig. 3C). The yeast utilized almost all available maltose within the first day of fermentation,

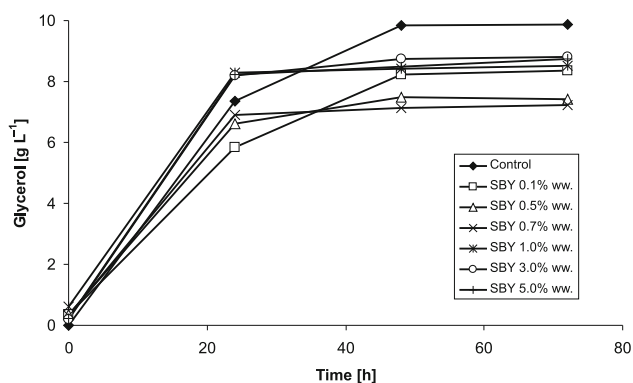
and its concentration did not change until the end of the process in all studied samples. Glucose concentration in the prepared mash ranged 80–90 g L<sup>-1</sup> (Fig. 3D). In the control sample, yeast utilized 50% of available glucose within the first day of fermentation. In the case of mash supplemented with SBY, the consumption of glucose was accelerated. Its concentration in the mash supplemented with 0.7% ww. and more of SBY was below 7 g L<sup>-1</sup> after the first day. Within subsequent days of fermentation yeast consumed almost all available glucose in the fermentation media. The calculated rate of sugars consumption (*R<sub>s</sub>*) and degree of attenuation (*α*) were also improved by the supplementation of mash with spent yeast (Table 1). The increased concentration of glucose oligomers in mash supplemented with SBY was probably caused by an increased viscosity (increased level of solids) during mashing what slightly affected the effects of enzymatic hydrolysis of starch what was observed previously<sup>18</sup>. An increase in the concentration of maltotriose within the first day of fermentation was caused by enzymatic saccharification of residual highly polymerized dextrins by enzymes added in the process of mashing, a similar phenomenon was observed earlier<sup>14</sup>. The accelerated glucose utilization during the fermentation of mash supplemented with SBY was caused by media enrichment in nutritional compounds. Laopaiboon and co-workers<sup>19</sup> proved that inorganic salts (ammonium sulphate) are not sufficient for fast sugar consumption, while complex media nutrients like yeast extract<sup>4</sup>, yeast hulls<sup>3</sup>, Casamino acids<sup>5</sup> or DDGS<sup>7</sup> lead to significant improvement of sugars utilization.

The production of ethanol (Fig. 4) and calculated kinetics of fermentation (Table 1) were improved in mash



**Figure 3.** Utilization of sugars (dextrins (DP4+) – A, maltotriose – B, maltose – C, glucose – D) during fermentation of corn mash supplemented with spent brewer's yeast (SBY)

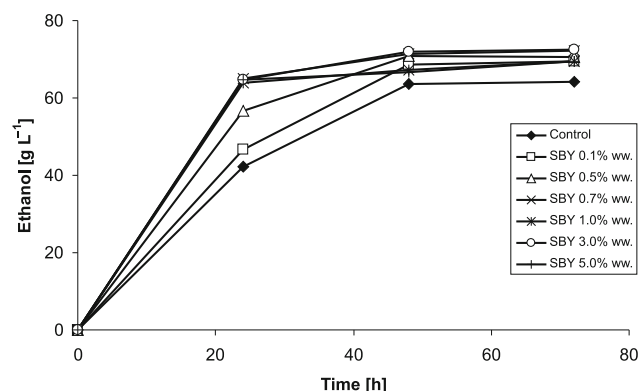
prepared with the addition of SBY. The concentration of ethanol after 24 h fermentation of mash supplemented with minimum of 0.7% ww. SBY was ca. 65 g L<sup>-1</sup> what corresponded with ethanol production rate of ca. 2.5 g L<sup>-1</sup>h<sup>-1</sup> while, after the same time in the control sample yeast produced only 42 g L<sup>-1</sup> of ethanol with 1.76 g L<sup>-1</sup>h<sup>-1</sup> rate. In the case of mash supplemented with 0.1 and 0.5% ww. of SBY the ethanol fermentation parameters were better than in the control sample but not as high as in media supplemented with 0.7% ww. and more of SBY. The practical ethanol yield after the completion of fermentation was improved by minimum 6.52% in the case of fermentation media supplemented with SBY. The highest ethanol yield (91.09% of theoretical) was obtained for the mash supplemented with 3.0% ww. of spent brewer's yeast. The improvement in ethanol formation rate by media supplementation was observed in previous study using inorganic salts, urea and yeast extract<sup>4</sup>.



**Figure 4.** Dynamics of ethanol production during fermentation of corn mash with addition of spent brewer's yeast (SBY)

There were found small amounts of glycerol (0.2–0.6 g L<sup>-1</sup>) in the mash supplemented with spent brewer's yeast cells (Fig. 5). SBY addition at 1.0% ww. and more improved formation of glycerol within first day of fermentation while lower doses caused reduction in glycerol production in comparison to control sample. The concentration of glycerol in fermenting mash supplemented with SBY did not significantly change by the end of fermentation, moreover the overall glycerol yield was lower in the supplemented media in comparison to control. Glycerol is produced by yeast as a response to high concentration of sugars at the beginning and ethanol at the end of fermentation<sup>20</sup>. Overproduction of glycerol is the cause for ethanol yield reduction so it should be avoided. In the studied fermentation processes lower concentrations of glycerol were obtained in fermentation

media supplemented with SBY. This was caused by an addition of excess assimilable nitrogen what reduces the formation of glycerol<sup>21</sup>. The initial presence of trace amounts of glycerol in the mash was probably caused by an extraction of intracellular glycerol from the spent yeast during the mashing process.



**Figure 5.** Dynamics of glycerol production during fermentation of corn mash supplemented with spent brewer's yeast (SBY)

## CONCLUSIONS

The experimental results confirmed that utilization of spent brewer's yeast for the supplementation of corn mash improves ethanol productivity and yield. Moreover, the time of fermentation could be reduced without the yield loss in comparison to the non-supplemented mash. The optimal dose of spent yeast proved to be 0.7% ww.

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**Table 1.** Kinetic parameters of ethanol fermentation of corn mash supplemented with spent brewer's yeast (SBY): Rs – rate of sugars consumption (in calculation to glucose),  $\alpha$  – degree of attenuation (in calculation to glucose), Rp – rate of ethanol production, Yp – practical ethanol yield

Variant	Rs [g L <sup>-1</sup> h <sup>-1</sup> ]			$\alpha$ [%]			Rp [g L <sup>-1</sup> h <sup>-1</sup> ]			Yp [%]		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Control	3.73	2.04	0.02	62.14	96.13	96.45	1.76	0.89	0.03	52.98	79.83	80.67
SBY, 0.1% w/w	4.20	1.73	0.26	66.53	93.93	98.02	1.95	0.92	0.03	58.72	86.46	87.40
SBY, 0.5% w/w	4.93	1.06	0.21	78.30	95.10	98.37	2.36	0.59	0.01	71.32	89.11	88.82
SBY, 0.7% w/w	5.94	0.48	0.08	90.17	97.39	98.64	2.71	0.26	0.04	81.81	89.67	90.88
SBY, 1.0% w/w	5.70	0.33	0.01	91.33	96.59	96.75	2.66	0.14	0.09	80.26	84.45	87.19
SBY, 3.0% w/w	5.35	0.46	0.01	88.81	96.50	96.65	2.70	0.30	0.02	81.41	90.51	91.09
SBY, 5.0% w/w	5.31	0.45	0.03	88.90	96.48	96.93	2.69	0.08	0.12	81.23	83.73	87.23



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